A Protein Biomarker Approach to Biodosimetry

FDA/CDRH Public Meeting
Regulatory Science Considerations for Medical Countermeasure Radiation Biodosimetry Devices
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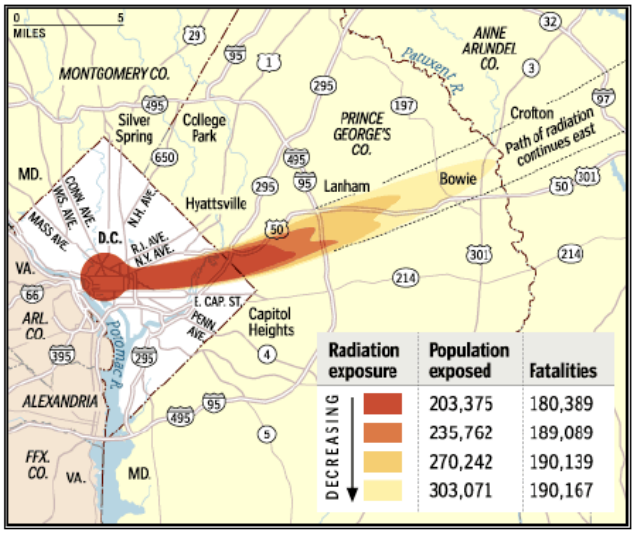
Diagnostics for Large-Scale Radiation Incidents

Scenario:

- Improvised nuclear device (10 kT) in metropolitan area
- Potential casualties from radiation exposure > 100,000
- Large numbers of injuries from blast effects and broken glass (2 mi radius)



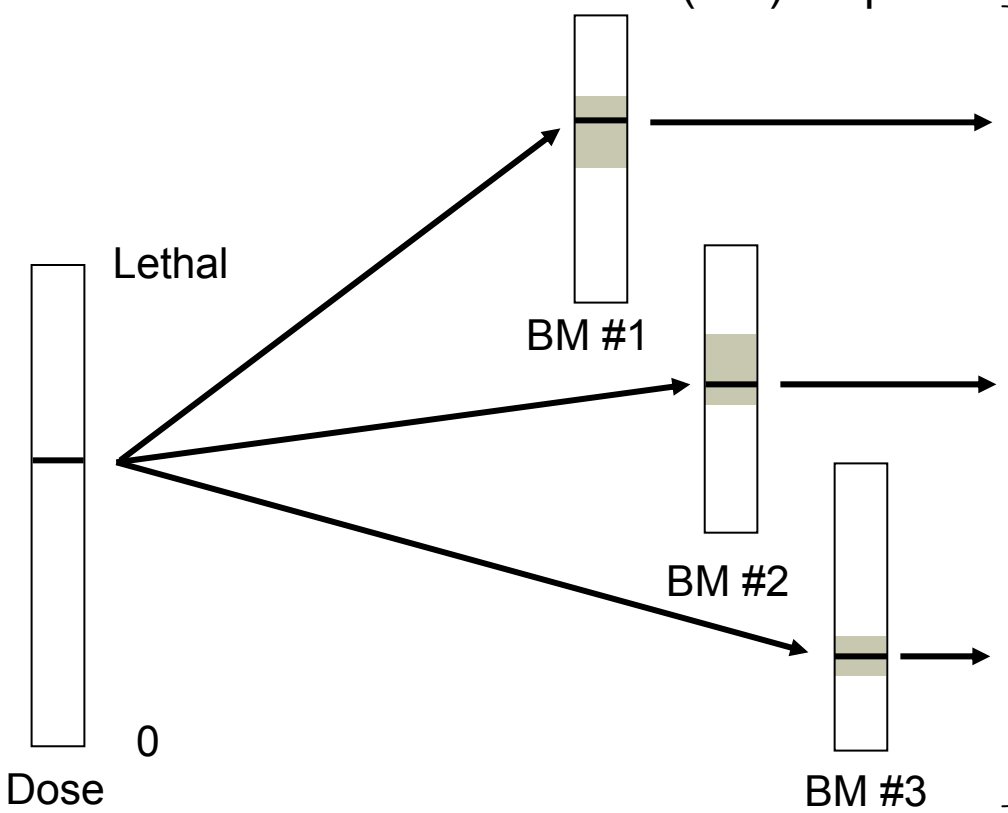
Washington Post, 2007

Need for Diagnostics (from BARDA BAA-BARDA-09-36):

- High-throughput (HT) and point-of-care (POC) tools that will:
  - Identify patients requiring urgent medical treatment
  - Provide assurance to individuals with low-dose exposure
  - Improve risk assessment for delayed effects of radiation exposure
  - Provide patient tracking
  - Potentially, monitor therapy and recovery
- Specific dose assessment needs:
  - Rapid classification of patients into low (< 2 Gy) and high (> 2 Gy) exposure
  - Quantitative assessment of dose between 0.5 and 10 Gy

Radiation Response and Biomarkers

Radiation exposure dose → Diagnostic information on physiological effect via biomarker (BM) response → Treatment decisions



Therapeutic strategy depends on evaluation of radiation-induced damage to physiological systems

Hematopoietic syndrome
Organ damage
Inflammation

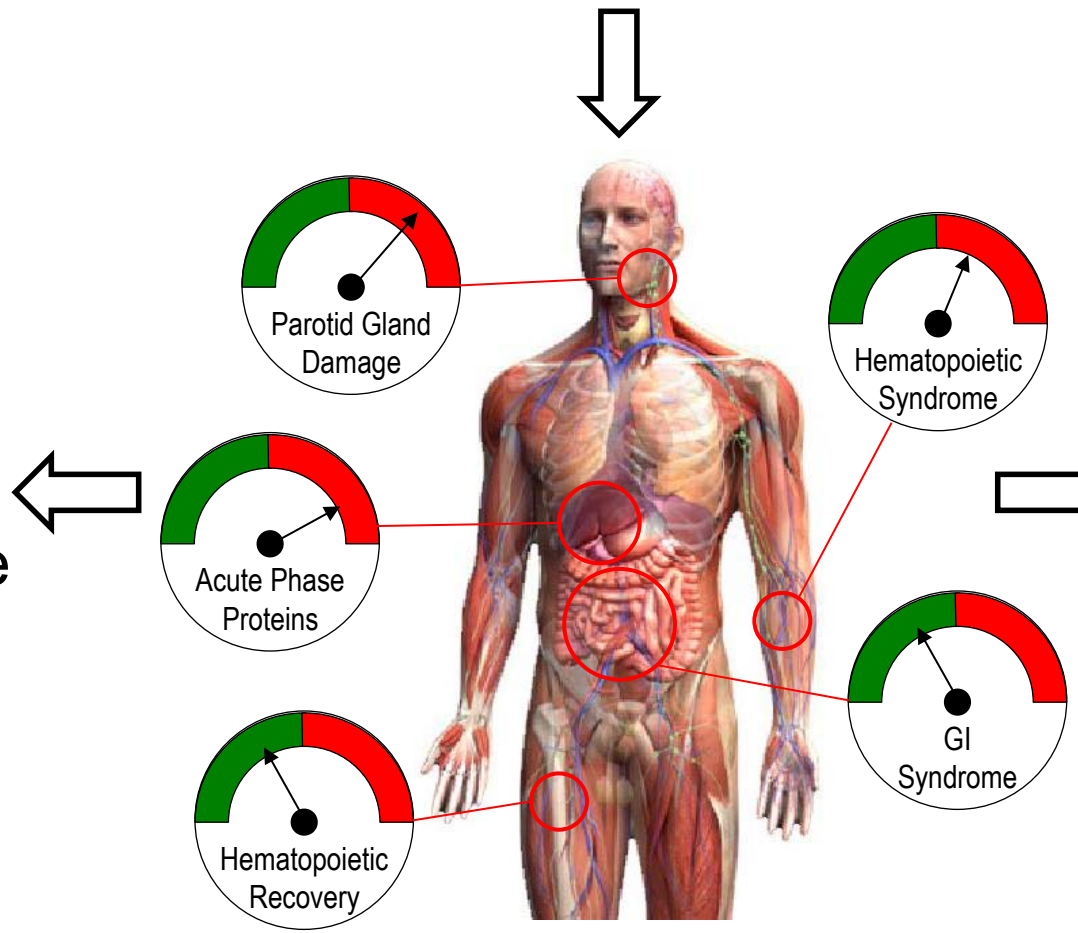
Protein biomarkers are closely linked to the state of physiological systems

Factors affecting biological response to radiation

- Individual susceptibility
- Heterogeneity of received dose

Biomarkers of Radiation Injury & Response

Measure Radiation Biomarkers



Estimate Dose of Exposure

Diagnose, Treat and Monitor Radiation Syndromes

MSD Biodosimetry Program

Protein Biomarker-Based Dosimetry

- Multi-parameter approach employs biomarkers associated with different mechanisms of radiation response
- Able to provide dose assessment over extended time period
- Provides information that is directly linked to physiological injury and treatment

Technology and Instrumentation

- State-of-the-art platform for multiplexed biomarker measurements in both laboratory (HT) and field (POC) settings
- Instrumentation designed for broad utility in medical countermeasures and commercial diagnostics – likely to be widely deployed in the future
- Offers a realistic solution to biodosimetry in response to a large-scale radiation event – with speed, throughput and accessibility

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Collaborating Laboratories:

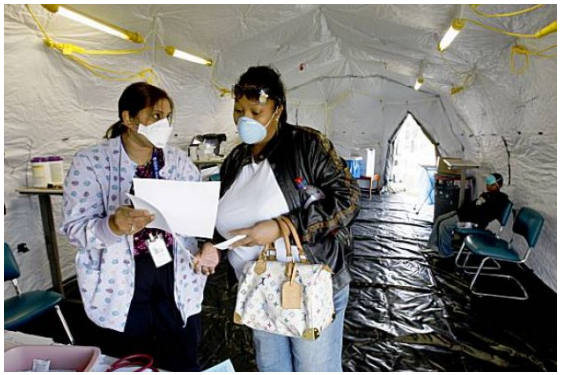
- Armed Forces Radiobiology Research Institute (AFRRI): Drs. Ossetrova and Blakely
- National Cancer Institute (NCI): Dr. Camphausen

Lab & POC: Common Biomarker Approach

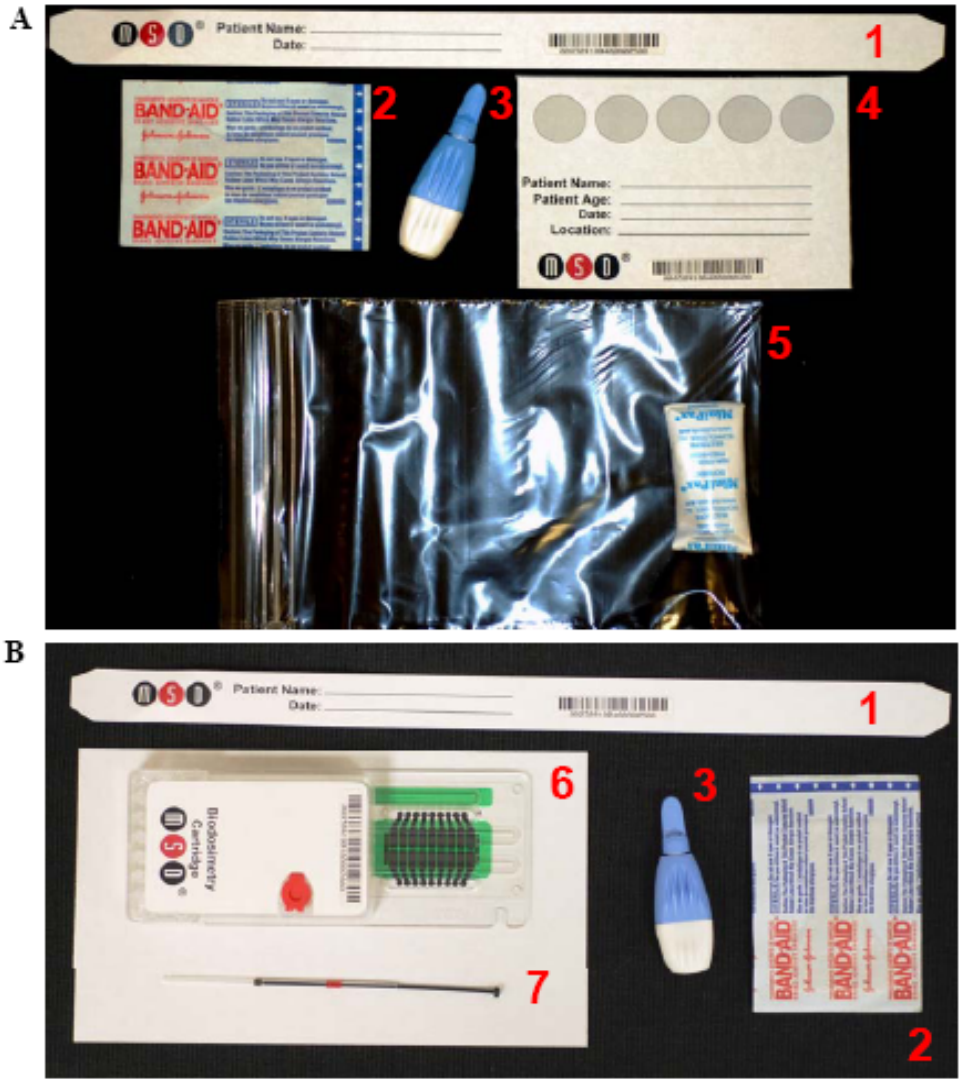
Multiplexed Biodosimetry Assay Panel

High Throughput Testing

POC Testing



Biodosimetry Sample Collection Kit Components



HT Kit

1. Bar-coded patient wrist band
2. Adhesive bandage
3. Single-use safety lancet
4. Dried blood spot collection card (with bar code)
5. Sample transport bag with desiccant

Note: Assay plates/reagents provided separately

POC Kit

6. Single-use POC test cartridge
7. Single-used capillary with plunger for blood transfer to cartridge

Approach to Biomarker Identification & Selection

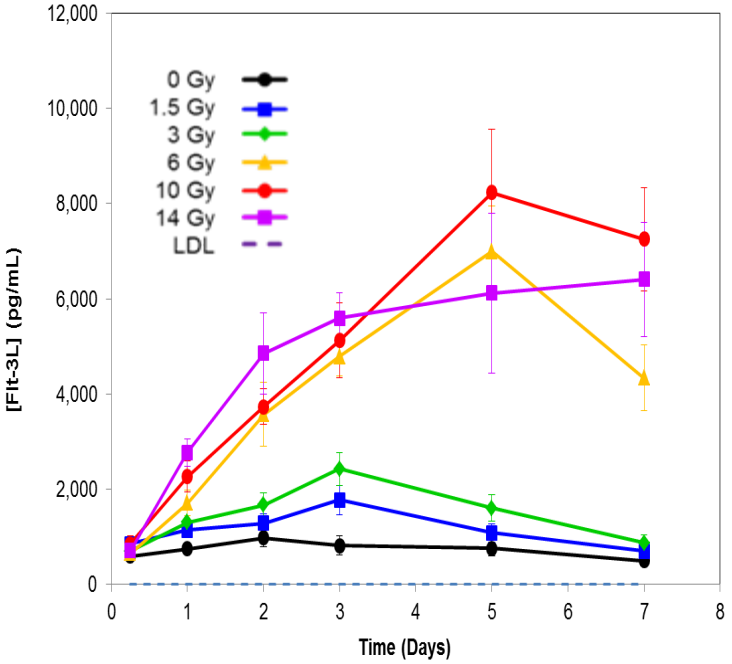
- Test known and proposed biomarkers of radiation response in animal models (mouse and NHP)
- Cover a wide range of potential physiological effects – hematopoietic system damage and recovery, tissue damage, inflammatory response, DNA damage, etc.
- Include cell surface proteins in plasma as surrogates for hematology measurements (cell counts)
- Identify radiation responsive markers, and evaluate combinations of markers in a dose-assessment algorithm
- Test dose-assessment algorithm in a blinded study (mouse model)

Biomarker Evaluation

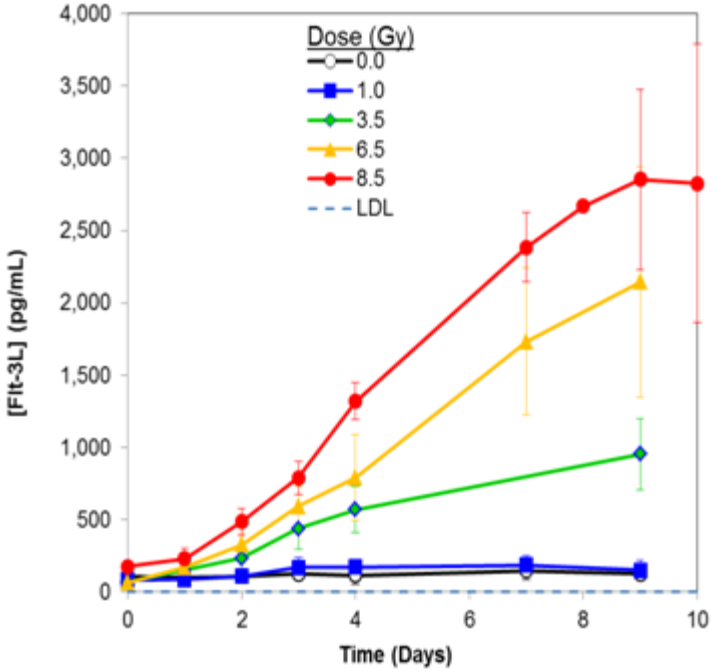
- Protein biomarkers were tested in mouse and NHP models of radiation response (> 50 candidates)
- For evaluation, biomarkers tested in plasma samples
- Biomarkers represented different categories of response:
  - DNA damage
  - Inflammatory response
  - Tissue damage (organ-specific)
  - Hematopoietic system injury and recovery
  - Protein surrogates for hematology measurements
- Selected biomarkers with clear radiation response, and minimal response to confounding injury (wound) in the mouse model

Example of Biomarker Response: Flt-3 Ligand

Mouse model



NHP model



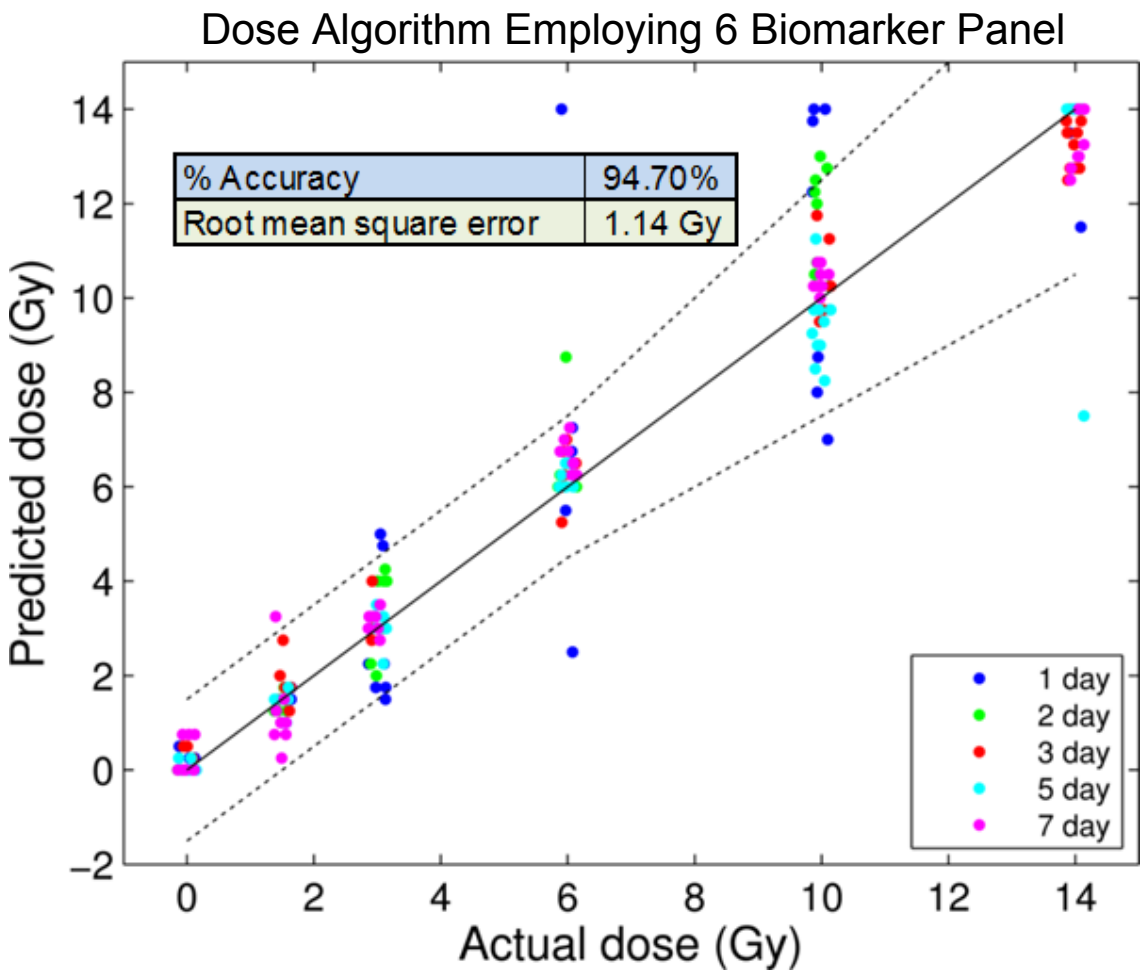
Dose equivalence for different species

Species/Strain	$LD_{50/30}$ or $LD_{50/60}$		Estimated Critical Threshold for ARS[4]
	Measured[1]	Ratio to Human	
Female B6D2F1/J Mice	9.3 Gy[2]	~2.5	~ 5 Gy
Rhesus Monkey	4.4-6.7 Gy[3]	~1.4	~ 3 Gy
Human	3-5 Gy[3]	1	2 Gy

1. X-ray or γ-ray with minimal care (mice, humans, rhesus) and mixed neutron and γ-ray with minimal or normal care (humans)
2. Ledney et al. (2010) Health Physics 98: 145-152.
3. DiCarlo AL et al. (2011) Disaster Med Pub Health Prep 5: S32-S44.
4. ARS = Acute Radiation Syndrome

Dose Assessment of Blinded Samples (Mouse Study)

- All mouse samples were blinded during testing.
- The predicted dose is shown as a function of the actual dose.
- Points for which the predicted dose exactly matches the actual dose will fall on the solid line.
- Points within the dashed lines meet our dose prediction accuracy criteria and are within 1.5 Gy of the actual dose (below 6 Gy) or within 25% of the actual dose (above 6 Gy).
- The inset shows the percentage of the predicted doses that fall within our accuracy criteria and the root mean square error in the predicted doses across the data set.



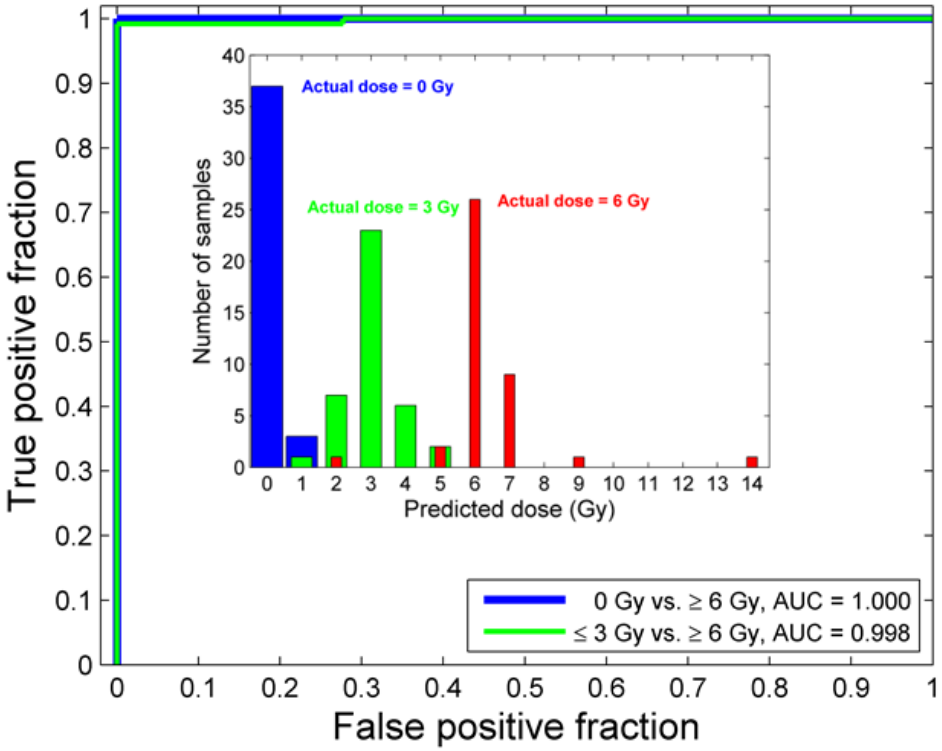
The panel provides excellent performance in assessing dose for blinded samples.

Algorithm Verification: Dose Classification of Blinded Samples

- Blinded study data was analyzed for the ability of the algorithm to classify dosage above or below the critical 2 Gy dose threshold in humans (~5 Gy in mice).
- ROC curve for distinguishing doses ≥ 6 Gy from non-irradiated controls is in blue.
- ROC curve for distinguishing doses ≥ 6 Gy from doses ≤ 3 Gy is in green.
- Inset (histogram) shows distribution of the predicted doses for samples receiving 0 Gy, 3 Gy or 6 Gy doses and demonstrates the separation of these distributions.
- Classification performance at the optimal predicted dose thresholds is provided in the table.

Near perfect discrimination of doses above and below critical threshold of 5 Gy (~2 Gy in humans)

Dose Algorithm Employing 6 Biomarker Panel



Classification Performance Using the Optimal Threshold		
Classification criteria	**0 Gy vs. ≥ 6 Gy**	**≤ 3 Gy vs. ≥ 6 Gy**
True positive fraction	1.0	0.992
True negative fraction	1.0	1.0
Prediction accuracy (%)	100	99.59
Area under curve (AUC)	1.000	0.998

Anticipated Challenges with Clinical Study

Human clinical samples

- Accidental exposure to high-dose total body irradiation (TBI) is rare, actual dose received is not accurately known, and 1-7 day samples are usually not available.
- Most medical radiation treatments use highly localized radiation with exposure limited to 1-2% of total body. These treatments do not produce the effects seen in TBI.
- TBI is sometimes used in medical practice (e.g. bone marrow transplants in blood cancers), but always in combination with chemotherapy. Very few clinical protocols treat with radiation prior to chemotherapy.
- When used medically, TBI is always fractionated into smaller doses to avoid radiation syndromes (e.g. 2 Gy fractions twice daily, or 1.25 Gy three times daily).

Development and validation of a biodosimetry test has to rely heavily on experimental animal studies, where the radiation dose can be carefully controlled and samples collected over a time course. FDA guidance on use of the Animal Rule for drugs/biologics can provide a starting point for designing studies.

A Potential Clinical/Regulatory Approach: Animal and Human Studies

Mouse Model: Developing and Validating Dose Algorithm, Testing Key Variables

- Identification of biomarkers
- Radiation dose and time response
- Algorithm development
- Effect of combined injury
- Radiation type (photon vs. mixed photon/neutron) and dose rate
- Partial body exposure vs. total body
- Age and gender study

NHP Model (Rhesus): Validating Biomarkers and Algorithm

- Dose studies for algorithm development and validation
- Studies on the effect of therapeutics (G-CSF) on biomarker levels

Humans: Connecting Results from Animal Studies to Humans

- Evaluate algorithm performance in patients receiving TBI for stem cell transfer
- If available, confirm in samples from TBI in radiation accidents (extremely rare)
- Demonstrate specificity with samples from individuals representative of US population

An Alternative Approach to Radiation Diagnostics?

Biodosimetry (Estimation of Received Dose)

- Advantages
  - Single easily interpretable output
  - Direct connection to physical dosimetry measurements
- Disadvantages
  - Not directly linked to patient health status
  - May not accurately reflect physiological injury (e.g. partial body exposure)
  - Difficult to achieve regulatory clearance: clinical trials are not feasible

Diagnosis of Specific Radiation Syndromes (Hematopoietic, GI, etc.)

- Advantages
  - Directly tied to patient health status
  - Provides an indication for treatment (e.g., G-CSF for hematopoietic syndrome)
  - Provides alternative approaches and patient populations for clinical trials (e.g., non-radiation related causes of myelosuppression)
- Disadvantages
  - No direct information on radiation dose
  - Different syndromes require different panels of biomarkers

A Possible Alternative Approach: Syndrome-Specific Diagnostics

Hematopoietic Syndrome and Other Myelosupressive Conditions

- The primary radiation pathology in the critical range for treatment (~2 to 6 Gy) is drop in blood cell counts from loss of bone marrow activity
- Other conditions associated with severe myelosuppression:
  - Side effect of chemotherapy for cancer treatment
  - Intended effect of myeloablative chemotherapy prior to stem cell replacement for blood cancers or other disorders
- Alternative strategy for clearing diagnostics for hematopoietic syndrome: Demonstrate ability of diagnostic to detect myeloablation associated with chemotherapy as a surrogate for radiation
  - Much larger accessible patient pool for clinical studies
  - Potential commercial applications in conventional medical care

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MSD

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